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Structure/Function Properties

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| <b>13. ABSTRACT (Maximum 200 Words)</b><br><p>The purpose of this research program is to contribute towards two major objectives in support of advancing our ability to prevent or treat bone failure or fragility:</p> <ol style="list-style-type: none"><li>1. Developing and characterizing methods of evaluating bone properties in animal models that goes beyond measures of bone density and global mechanical properties.</li><li>2. Evaluating the influence of physical forces and nutritional status on bone biomechanical integrity.</li></ol> <p>Specifically, it was the purpose of this study to apply a hierarchical approach to quantifying the properties of murine bone to the level of the extracellular matrix. Furthermore, the study was designed to test hypotheses concerning the interplay between vitamin D and calcium nutritional support and physical forces.</p> <p>Progress during the third year has followed the proposed statement of work. 394 animals have been completed and nearly all the vertebra, femurs and tibias have been evaluated geometrically, morphologically and mechanically. Genetic strain has a significant influence on properties, but exercise has had little effect. Nutrition is also important. From a histologic perspective, the tissue constituency is unaffected, suggesting only geometric variations, not inherent material changes.</p> |                                                                 |                                                                |                                                                               |                                  |
| <b>14. SUBJECT TERMS</b><br>Bone biomechanics, mechanotransduction, nutrition, fragility, osteoporosis                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                 |                                                                |                                                                               | <b>15. NUMBER OF PAGES</b><br>22 |
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## **A. Introduction**

It is well known that the maintenance and adaptation of bone integrity is dependent on a complex interaction of metabolic and environmental factors (mechanical stresses, nutritional status). Unfortunately, the specific relationship between these factors and the biomechanical competence of bone tissue remains incompletely quantified. As a result, strategies for preventing or effectively treating bone fragility or enhancing general bone health are far from being optimized. The specific goals of this research program is to contribute to two major objectives in support of reducing the incidence of fracture:

- a. The development and application of micro-imaging and testing techniques in animal models to study bone structure function properties.
- b. Exploring the influence of calcium and vitamin D metabolism and physical forces on bone integrity.

## **B. Body**

The progress of this research program is described below, as a function of the statements of work that were approved by the USAMRMC. The statement of work was proposed as follows:

1. The acquisition of DBP founder mice and breeding will be performed during year 1 and year 2 to produce 180 animals for testing.
2. Mechanical fabrication and calibration of all testing holders and test fixtures will be completed during the first 9 months of study. Maintenance, recalibration and replacement of parts will continue year 2 through year 4.
3. Micro CT, whole bone testing of DBP mice will be completed year 1 and year 2.
4. Microspecimen production and testing of DBP bones will be completed year 2 through year 3.
5. Micro CT, Whole bone testing of C57BL/6J and C3H/HeJ bones will be conducted year 1 through year 3.
6. Microspecimen testing of C57BL/6J and C3H/HeJ bone will be tested year 2.5 through year 3.5.
7. Raman imaging, SEM, and light microscopy of DBP mice bone will be conducted year 1 through year 3.
8. Raman imaging, SEM, and light microscopy of C57BL/6J and C3H/HeJ bone will be tested year 2 through year 3.5.
9. Final data analyses and correlations across all groups will be completed during year 4.

Since many of the tasks were described as objectives to be completed over 1 to 4 years, the progress report can't follow these nine tasks precisely. Instead, we have presented the specific tasks that were proposed for completion during the third year. The tasks are outlined in "**bold**", followed by a description of the accomplishments.

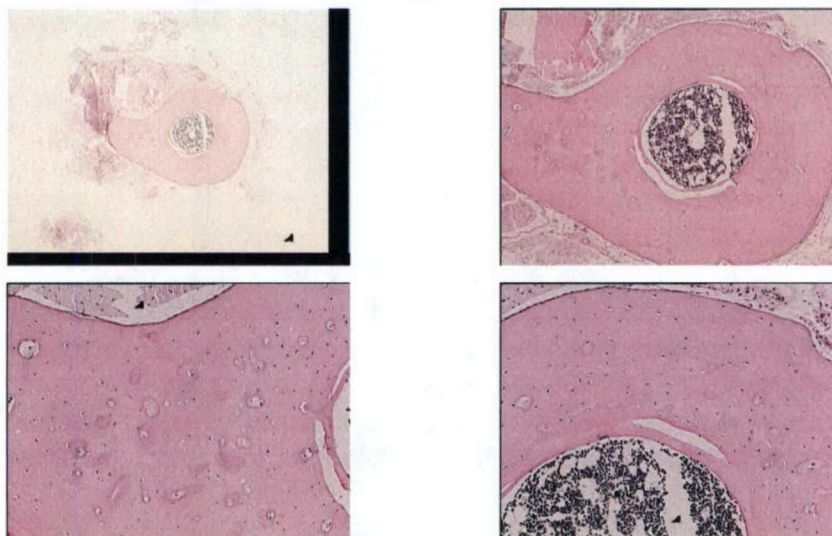
### **The acquisition of DBP founder mice and breeding will be performed to produce the 180 mice for testing**

As noted in earlier progress reports, DBP breeder mice (4 females and 2 males) were obtained from Dr. Nancy Cooke at the University of Pennsylvania. These mice are being used to generate our own population of heterozygote breeders. Due to background noise, homozygous breeders cannot be used. We have also developed protocols for genotyping the newborn mice.

During the past year we have worked, feverishly to breed these mice, but have continued to run into some difficult problems. We lost most of our colony to an outbreak of MPV (Mouse Parvo Virus) that afflicted many areas in our institution. We also had some difficulties with infertility. We have risen above most of these problems and have the colony growing and being entered into the testing protocols. As a result we have done some adjustments in our overall schedule and completed additional testing activity on other mouse bones, thereby maintaining the overall excellent progress and providing some flexibility to complete the breeding and testing. We are confident that we will still meet our overall objectives in the program. The goal of examining the DBP mice has been rescheduled to be completed during the next 15 months.

### **Histologic assessment of the mouse bone constituency**

As proposed, we have prepared and reviewed the histologic features of selected bone specimens from all of our animal groups evaluated to date. This analysis was performed to assess whether there were microanatomical or morphologic features that varied among the test groups. We prepared and analyzed 120 bones. These procedures went very well and we determined that there were no systematic variations in matrix content, cell morphology, cell matrix interactions and other features. An example of the histologic sections and review is illustrated in Figure 1.



**Figure 1:** Example of histologic features of murine bone samples. No systematic differences were observed in any of the group.

### **Micro CT and whole bone testing of bones will be conducted year 1 through year 3**

All acquisition, Micro CT and whole bone mechanical testing is now near completion for all the mice, excluding the DBP colony. This represents the largest portion of the proposed program and also represents the majority of progress for the program. We have only 240 femurs remaining to test, and all of the vertebral bodies have now been tested. The vertebral studies represent a substantial accomplishment for this period. By way of summary, we have completed the following:

#### **A. Femoral Analyses**

| <b>Studies</b>        | <b>Micro CT scan</b> | <b>Micro CT analysis</b> | <b>Mechanical testing</b> | <b>Raman spectroscopy</b> |
|-----------------------|----------------------|--------------------------|---------------------------|---------------------------|
| Completed             | 392                  | 392                      | 128                       | 30                        |
| Remaining to complete | 0                    | 0                        | 240                       | 100                       |

#### **B. Vertebral Body Analyses**

| <b>Studies</b>        | <b>Micro CT scan</b> | <b>Micro CT analysis</b> | <b>Mechanical testing</b> |
|-----------------------|----------------------|--------------------------|---------------------------|
| Completed             | 382                  | 381                      | 394                       |
| Remaining to complete | 0                    | 0                        | 0                         |

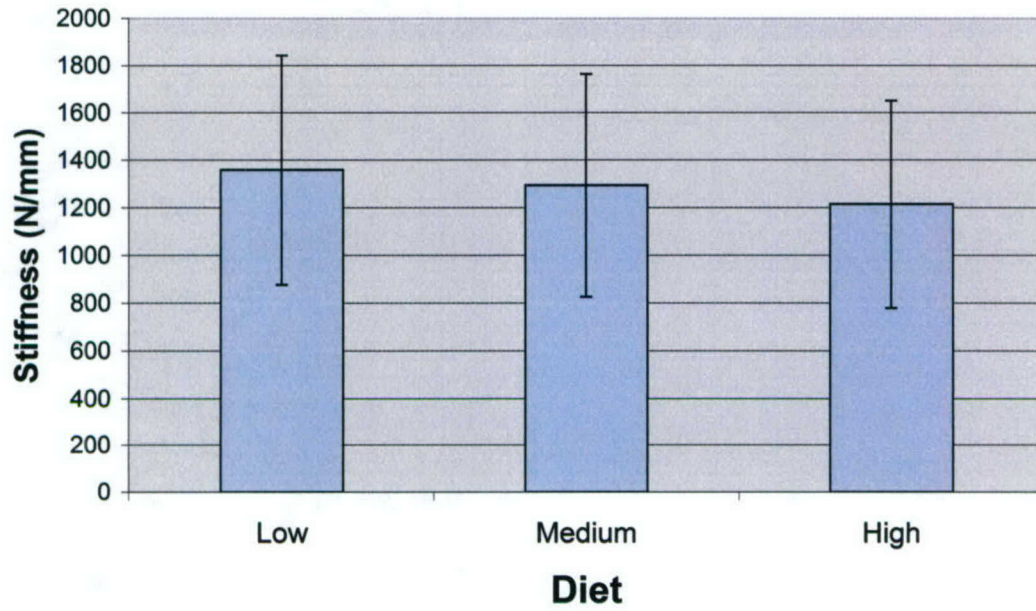
#### **Microspecimen preparations**

We have now completed the protocols for the preparation and machining of all of the microspecimens for ECM material property evaluations. This time consuming process has been optimized using the micro-milling system we have fabricated and associated dissecting microscope imaging. We have completed the machining of approximately 30 bone segments to date. We have also completed the validation of the micro-mechanical testing systems using both 4 point bending and nanoindentation.

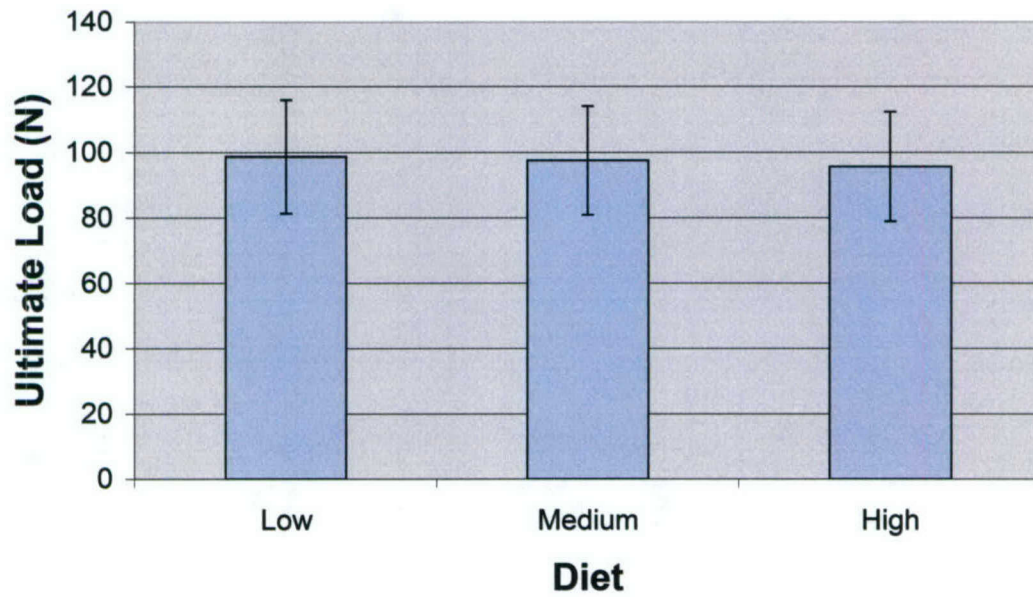
#### **Example results for this period**

The results from analysis of the vertebral body mechanical tests can be seen from the following graphs. These have been segmented into effects by diet and effects by exercise.

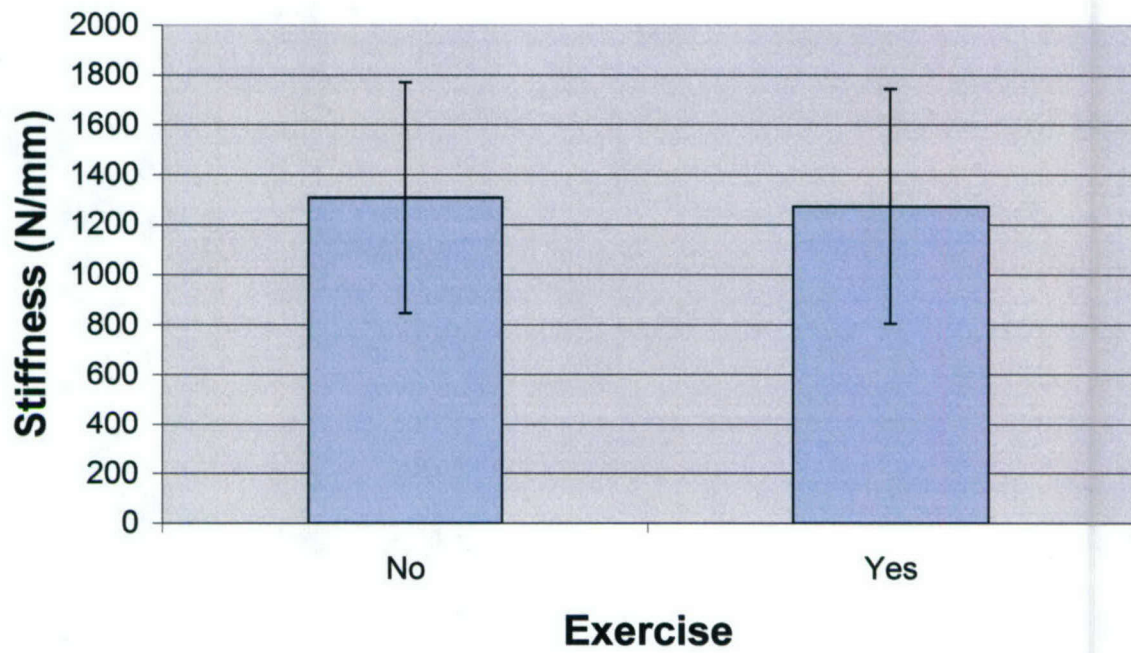
**Vertebral Stiffness by Diet**



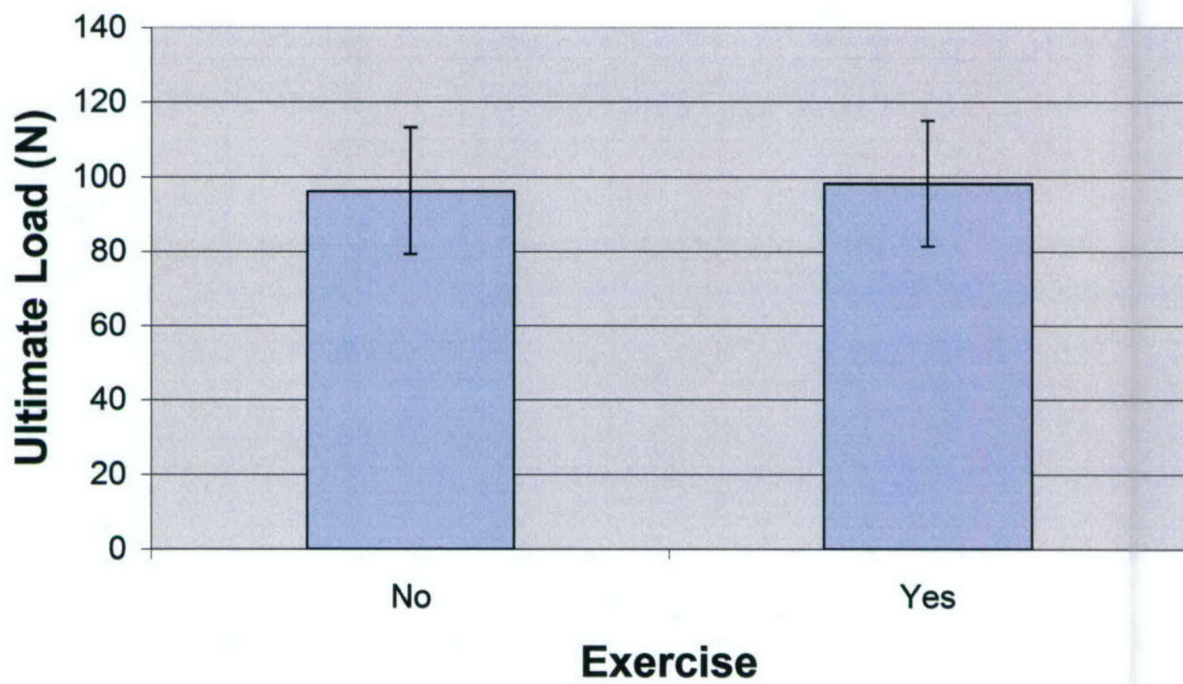
**Vertebral Ultimate Load by Diet**



### Vertebral Stiffness by Exercise



### Vertebral Ultimate Load by Exercise



### **C. Key Research Finding**

- Micro-milling and micro-testing systems fabricated are functioning and specimens are currently under production.
- 394 mice, which were entered into study to evaluate effects of gender, strain, nutrition and exercise on morphology and mechanical properties of bone, have been studied. All have been analyzed for vertebral mechanical and morphologic properties. All femoral specimens have been analyzed for morphology using Micro CT and 128 have been completed for mechanical testing.
- 120 tibial specimens have been prepared for histologic analysis. All have been sectioned, stained analyzed and documented using a Bioquant imaging system.
- DBP mice colony is being reconstituted and will be evaluated for properties during the next 15 months. These tasks have been rescheduled due to problems with breeding and a viral outbreak. Shifting of other tasks and procedures has been accomplished to maintain the overall schedule of the program.
- Mouse strain had a significant effect on geometric and preliminarily mechanical properties in the femurs. Strain had a significant effect on vertebral properties.
- Exercise did not have a significant effect on any of the femoral or vertebral mechanical whole-bone parameters.
- In vertebra, the normal dietary calcium group had a significantly higher bone volume fraction as compared to the low ( $p=0.003$ ) and high ( $p=0.003$ ) groups. The bone surface to volume ratio of the normal group was shown to be significantly lower than the low dietary calcium group ( $p=0.030$ ), however no significant differences were seen between any of the other groupings.
- In vertebra, the normal dietary calcium group had a significantly higher trabecular thickness as compared to the low group ( $p<0.000$ ) and marginally significant as compared to the high ( $p=0.051$ ) group.
- Exercise has had no effect on the parameters tested to date. It may be possible that the material properties are affected, but we would hypothesize that these parallel the whole bone properties.

### **D. Reportable Outcomes**

Kriegel JM, Oyserman S, Roller SA, Blumenfeld J, Volkman SK, Nashi S, Hall JM, McCreddie BR, Goldstein SA: Influences of nutrition and physical forces on bone structure/function properties. Trans ORS 50th Annual Meeting, No: 0393, March 7-11, 2004, San Francisco, CA.

### **E. Conclusions**

The third year of work has been very successful and productive, despite the setback with DBP breeding. We have entered and analyzed a very large number of animals in to the study and have maintained the timetable originally proposed in the program. The first data of femoral and vertebral bone demonstrate significant effects of mouse strain and dietary calcium levels. The studies have also demonstrated the ability of the micro-imaging and testing protocols to determine the effects of a variety of factors on bone structure and function properties.

**F. References**

None

**G. Appendices**

1. Mouse census database for all animals entered into the studies.
2. Abstract

Database documenting the animals entered into the studies and progress

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet | Exercise | Femur |        |       |       | Vertebrae |        |       |        | Tibia     |         |
|----------|---------|-------|------|-----|--------|---------|------|----------|-------|--------|-------|-------|-----------|--------|-------|--------|-----------|---------|
|          |         |       |      |     |        |         |      |          | Scan  | Analy. | Mech. | Raman | Scan      | Analy. | Mech. | Analy. | Histology | Re-fill |
| 2        | 1a      | 1a    | R    | M   | C57    | 4/21/02 | Low  | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 1         | 0       |
| 9        | 1b      | 1b    | L    | M   | C57    | 4/21/02 | Low  | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 1       |
| 16       | 1c      | 1c    | B    | M   | C57    | 4/21/02 | Low  | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 23       | 1d      | 1d    | N    | M   | C57    | 4/21/02 | Low  | Yes      | 0     | 0      | 0     | 0     | 0         | 0      | 0     | 0      | 0         | 0       |
| 30       | 2       | 2     | R    | M   | C57    | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 37       | 2       | 2     | L    | M   | C57    | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 44       | 2       | 2     | B    | M   | C57    | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 51       | 2       | 2     | N    | M   | C57    | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 58       | 3       | 3     | R    | F   | C57    | 4/21/02 | Norm | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 65       | 3       | 3     | L    | F   | C57    | 4/21/02 | Norm | Yes      | 1     | 1      | 1     | 0     | 0         | 0      | 0     | 1      | 0         | 0       |
| 72       | 3       | 3     | B    | F   | C57    | 4/21/02 | Norm | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 79       | 3       | 3     | N    | F   | C57    | 4/21/02 | Norm | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 86       | 4       | 4     | R    | F   | C57    | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 93       | 4       | 4     | L    | F   | C57    | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 100      | 4       | 4     | B    | F   | C57    | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 107      | 4       | 4     | N    | F   | C57    | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 114      | 5a      | 5a    | R    | M   | G57    | 4/21/02 | High | Yes      | 0     | 0      | 0     | 0     | 0         | 0      | 0     | 0      | 0         | 0       |
| 121      | 5b      | 5b    | L    | M   | G57    | 4/21/02 | High | Yes      | 0     | 0      | 0     | 0     | 0         | 0      | 0     | 0      | 0         | 0       |
| 128      | 5c      | 5c    | B    | M   | C57    | 4/21/02 | High | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 1         | 0       |
| 135      | 5d      | 5d    | N    | M   | G57    | 4/21/02 | High | Yes      | 0     | 0      | 0     | 0     | 0         | 0      | 0     | 0      | 0         | 0       |
| 142      | 6       | 6     | R    | M   | C57    | 4/21/02 | High | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 149      | 6       | 6     | L    | M   | C57    | 4/21/02 | High | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 156      | 6       | 6     | B    | M   | C57    | 4/21/02 | High | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 163      | 6       | 6     | N    | M   | C57    | 4/21/02 | High | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 170      | 7       | 7     | R    | M   | C3     | 4/21/02 | Low  | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 177      | 7       | 7     | L    | M   | C3     | 4/21/02 | Low  | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 184      | 7       | 7     | B    | M   | C3     | 4/21/02 | Low  | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 191      | 7       | 7     | N    | M   | C3     | 4/21/02 | Low  | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 198      | 8       | 8     | R    | M   | C3     | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 205      | 8       | 8     | L    | M   | C3     | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 212      | 8       | 8     | B    | M   | C3     | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 219      | 8       | 8     | N    | M   | C3     | 4/21/02 | Low  | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 226      | 9       | 9     | R    | F   | C3     | 4/21/02 | Norm | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 233      | 9       | 9     | L    | F   | C3     | 4/21/02 | Norm | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 240      | 9       | 9     | B    | F   | C3     | 4/21/02 | Norm | Yes      | 0     | 0      | 0     | 0     | 0         | 0      | 0     | 0      | 0         | 0       |
| 247      | 9       | 9     | N    | F   | C3     | 4/21/02 | Norm | Yes      | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 254      | 10      | 10    | R    | F   | C3     | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 261      | 10      | 10    | L    | F   | C3     | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 1         | 0       |
| 268      | 10      | 10    | B    | F   | C3     | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |
| 275      | 10      | 10    | N    | F   | C3     | 4/21/02 | Norm | No       | 1     | 1      | 1     | 0     | 1         | 1      | 1     | 1      | 0         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 282      | 11      | 1a    | R    | M   | C3     | 4/21/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 289      | 11      |       | L    | M   | C3     | 4/21/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 296      | 11      |       | B    | M   | C3     | 4/21/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 303      | 11      |       | N    | M   | C3     | 4/21/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 310      | 12      | 1b    | R    | M   | C3     | 4/21/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 317      | 12      |       | L    | M   | C3     | 4/21/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 324      | 12      |       | B    | M   | C3     | 4/21/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 331      | 12      |       | N    | M   | C3     | 4/21/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 338      | 13      | 2a    | R    | F   | C57    | 4/28/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 345      | 13      |       | L    | F   | C57    | 4/28/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 352      | 13      |       | B    | F   | G57    | 3/73/74 | Low  | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 359      | 13      |       | N    | F   | C57    | 4/28/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 366      | 14      | 2b    | R    | F   | C57    | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 373      | 14      |       | L    | F   | C57    | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 380      | 14      |       | B    | F   | C57    | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 387      | 14      |       | N    | F   | C57    | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 394      | 15a     | 2a    | R    | M   | C57    | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 401      | 15b     |       | L    | M   | C57    | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 408      | 15c     |       | B    | M   | C57    | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 415      | 15d     |       | N    | M   | C57    | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 422      | 16      | 2b    | R    | M   | C57    | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 429      | 16      |       | L    | M   | C57    | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 436      | 16      |       | B    | M   | C57    | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 443      | 16      |       | N    | M   | C57    | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 450      | 17      | 2a    | R    | F   | C57    | 4/28/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 457      | 17      |       | L    | F   | C57    | 4/28/02 | High | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 464      | 17      |       | B    | F   | C57    | 4/28/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 471      | 17      |       | N    | F   | C57    | 4/28/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 478      | 18      | 2b    | R    | F   | C57    | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 485      | 18      |       | L    | F   | C57    | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 492      | 18      |       | B    | F   | C57    | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 499      | 18      |       | N    | F   | C57    | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 506      | 19      | 2a    | R    | F   | C3     | 4/28/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 513      | 19      |       | L    | F   | C3     | 4/28/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 520      | 19      |       | B    | F   | C3     | 4/28/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 527      | 19      |       | N    | F   | C3     | 4/28/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 534      | 20      | 2b    | R    | F   | C3     | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 541      | 20      |       | L    | F   | C3     | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 548      | 20      |       | B    | F   | C3     | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 555      | 20      |       | N    | F   | C3     | 4/28/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 562      | 21      | 2a    | R    | M   | C3     | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 569      | 21      |       | L    | M   | C3     | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 576      | 21      |       | B    | M   | C3     | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 583      | 21      |       | N    | M   | C3     | 4/28/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|------|----------|------|--------|-------|-------|------|--------|-------|-----------|---------|
| 590      | 22      | 2b    | R    | M   | C3     | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 1       |
| 597      | 22      |       | L    | M   | C3     | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 604      | 22      |       | B    | M   | C3     | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 611      | 22      |       | N    | M   | C3     | 4/28/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 618      | 23      | 2a    | R    | F   | C3     | 4/28/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 1       |
| 625      | 23      |       | L    | F   | C3     | 4/28/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 1       |
| 632      | 23      |       | B    | F   | C3     | 4/28/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 1       |
| 639      | 23      |       | N    | F   | C3     | 4/28/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 646      | 24      | 2b    | R    | F   | C3     | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 1    | 1      | 1     | 1         | 0       |
| 653      | 24      |       | L    | F   | C3     | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 1       |
| 660      | 24      |       | B    | F   | C3     | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 667      | 24      |       | N    | F   | C3     | 4/28/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 1       |
| 674      | 25a     | 3a    | R    | M   | C57    | 7/17/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 681      | 25b     |       | L    | M   | C57    | 7/17/02 | Low  | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0         | 0       |
| 688      | 25c     |       | B    | M   | C57    | 7/17/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 695      | 25d     |       | N    | M   | C57    | 7/17/02 | Low  | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0         | 0       |
| 702      | 26      | 3b    | R    | M   | C57    | 7/17/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 709      | 26      |       | L    | M   | C57    | 7/17/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 716      | 26      |       | B    | M   | C57    | 7/17/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 723      | 26      |       | N    | M   | C57    | 7/17/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 730      | 27      | 3a    | R    | F   | C57    | 7/17/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 737      | 27      |       | L    | F   | C57    | 7/17/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 744      | 27      |       | B    | F   | C57    | 7/17/02 | Norm | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 751      | 27      |       | N    | F   | C57    | 7/17/02 | Norm | Yes      | 0    | 0      | 0     | 0     | 0    | 1      | 1     | 1         | 0       |
| 758      | 28      | 3b    | R    | F   | C57    | 7/17/02 | Norm | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0         | 0       |
| 765      | 28      |       | L    | F   | C57    | 7/17/02 | Norm | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 772      | 28      |       | B    | F   | C57    | 7/17/02 | Norm | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0         | 0       |
| 779      | 28      |       | N    | F   | C57    | 7/17/02 | Norm | No       | 1    | 1      | 1     | 1     | 1    | 1      | 1     | 1         | 0       |
| 786      | 29a     | 3a    | R    | M   | C57    | 7/17/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 793      | 29b     |       | L    | M   | C57    | 7/17/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 800      | 29c     |       | B    | M   | C57    | 7/17/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 807      | 29d     |       | N    | M   | C57    | 7/17/02 | High | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 814      | 30      | 3b    | R    | M   | C57    | 7/17/02 | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0         | 0       |
| 821      | 30      |       | L    | M   | C57    | 7/17/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 828      | 30      |       | B    | M   | C57    | 7/17/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 835      | 30      |       | N    | M   | C57    | 7/17/02 | High | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 842      | 31      | 4a    | R    | F   | C57    | 7/24/02 | Low  | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0         | 0       |
| 849      | 31      |       | L    | F   | C57    | 7/24/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 856      | 31      |       | B    | F   | C57    | 7/24/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 1    | 1      | 1     | 1         | 0       |
| 863      | 31      |       | N    | F   | C57    | 7/24/02 | Low  | Yes      | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 870      | 32      | 4b    | R    | F   | C57    | 7/24/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 877      | 32      |       | L    | F   | C57    | 7/24/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 884      | 32      |       | B    | F   | C57    | 7/24/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |
| 891      | 32      |       | N    | F   | C57    | 7/24/02 | Low  | No       | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain   | DOB  | Diet | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|----------|------|------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 898      | 33a 4a  | R     | M    | C57 | 7/24/02  | Norm | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 905      | 33b     | L     | M    | C57 | 7/24/02  | Norm | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 912      | 33c     | B     | M    | C57 | 7/24/02  | Norm | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 919      | 33d     | N     | M    | C57 | 7/24/02  | Norm | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 926      | 34 4b   | R     | M    | C57 | 7/24/02  | Norm | No   | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 933      | 34      | L     | M    | C57 | 7/24/02  | Norm | No   | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 940      | 34      | B     | M    | C57 | 7/24/02  | Norm | No   | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 947      | 34      | N     | M    | C57 | 7/24/02  | Norm | No   | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 954      | 35 4a   | R     | F    | C57 | 7/24/02  | High | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 961      | 35      | L     | F    | C57 | 7/24/02  | High | Yes  | 1        | 1    | 1      | 1     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 968      | 35      | B     | F    | C57 | 7/24/02  | High | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 975      | 35      | N     | F    | C57 | 7/24/02  | High | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 982      | 36 4b   | R     | F    | C57 | 7/24/02  | High | No   | 1        | 1    | 1      | 1     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 989      | 36      | L     | F    | C57 | 7/24/02  | High | No   | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 1       |
| 996      | 36      | B     | F    | C57 | 7/24/02  | High | No   | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1003     | 36      | N     | F    | C57 | 7/24/02  | High | No   | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1010     | 37a 4a  | R     | F    | C57 | 7/24/02  | High | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 0      | 0     | 1      | 0         | 0       |
| 1017     | 37b     | L     | F    | C57 | 7/24/02  | High | Yes  | 1        | 1    | 1      | 1     | 1     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1024     | 38 5b   | R     | M    | C57 | 10/28/02 | Low  | No   | 1        | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1031     | 38      | L     | M    | C57 | 10/28/02 | Low  | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1038     | 38      | B     | M    | C57 | 10/28/02 | Low  | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1045     | 38      | N     | M    | C57 | 10/28/02 | Low  | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1052     | 39a 5a  | R     | M    | C57 | 10/28/02 | Low  | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1059     | 39b     | L     | M    | C57 | 10/28/02 | Low  | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1066     | 39c     | B     | M    | C57 | 10/28/02 | Low  | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1073     | 39d     | N     | M    | C57 | 10/28/02 | Low  | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1080     | 40 5b   | R     | F    | C57 | 10/28/02 | Norm | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1087     | 40      | L     | F    | C57 | 10/28/02 | Norm | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1094     | 40      | B     | F    | C57 | 10/28/02 | Norm | No   | 1        | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1101     | 40      | N     | F    | C57 | 10/28/02 | Norm | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1108     | 41 5a   | R     | F    | C57 | 10/28/02 | Norm | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1115     | 41      | L     | F    | C57 | 10/28/02 | Norm | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1122     | 41      | B     | F    | C57 | 10/28/02 | Norm | Yes  | 0        | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1129     | 41      | N     | F    | C57 | 10/28/02 | Norm | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1136     | 42 5b   | R     | M    | C57 | 10/28/02 | High | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1143     | 42      | L     | M    | C57 | 10/28/02 | High | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1150     | 42      | B     | M    | C57 | 10/28/02 | High | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1157     | 42      | N     | M    | C57 | 10/28/02 | High | No   | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1164     | 43a 5a  | R     | M    | C57 | 10/28/02 | High | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1171     | 43b     | L     | M    | C57 | 10/28/02 | High | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1178     | 43c     | B     | M    | C57 | 10/28/02 | High | Yes  | 0        | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1185     | 43d     | N     | M    | C57 | 10/28/02 | High | Yes  | 1        | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB      | Diet | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|----------|------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 1192     | 44      | 5b    | R    | M   | C3     | 10/28/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1199     | 44      |       | L    | M   | C3     | 10/28/02 | Low  | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1206     | 44      |       | B    | M   | C3     | 10/28/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1213     | 44      |       | N    | M   | C3     | 10/28/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1220     | 45      | 5a    | R    | M   | C3     | 10/28/02 | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1227     | 45      |       | L    | M   | C3     | 10/28/02 | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1234     | 45      |       | B    | M   | C3     | 10/28/02 | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1241     | 45      |       | N    | M   | C3     | 10/28/02 | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1248     | 46      | 5b    | R    | F   | C3     | 10/28/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1255     | 46      |       | L    | F   | C3     | 10/28/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1262     | 46      |       | B    | F   | C3     | 10/28/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1269     | 46      |       | N    | F   | C3     | 10/28/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1276     | 47      | 5a    | R    | F   | C3     | 10/28/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1283     | 47      |       | L    | F   | C3     | 10/28/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1290     | 47      |       | B    | F   | C3     | 10/28/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1297     | 47      |       | N    | F   | C3     | 10/28/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1304     | 48      | 5b    | R    | M   | C3     | 10/28/02 | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1311     | 48      |       | L    | M   | C3     | 10/28/02 | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1318     | 48      |       | B    | M   | C3     | 10/28/02 | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1325     | 48      |       | N    | M   | C3     | 10/28/02 | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1332     | 49      | 5a    | R    | M   | C3     | 10/28/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1339     | 49      |       | L    | M   | C3     | 10/28/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1346     | 49      |       | B    | M   | C3     | 10/28/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1353     | 49      |       | N    | M   | C3     | 10/28/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1360     | 50a     | 5b    | R    | F   | C57    | 10/28/02 | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1367     | 50a     |       | N    | F   | C57    | 10/28/02 | High | No       | 1    | 1      | 0     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1374     | 50b     |       | R    | F   | C3     | 10/28/02 | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1381     | 50b     |       | N    | F   | C3     | 10/28/02 | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1388     | 51      | 6a    | R    | F   | C57    | 11/4/02  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1395     | 51      |       | L    | F   | C57    | 11/4/02  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1402     | 51      |       | B    | F   | C57    | 11/4/02  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1409     | 51      |       | N    | F   | C57    | 11/4/02  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1416     | 52      | 6b    | R    | M   | C57    | 11/4/02  | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1423     | 52      |       | L    | M   | C57    | 11/4/02  | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1430     | 52      |       | B    | M   | C57    | 11/4/02  | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1437     | 52      |       | N    | M   | C57    | 11/4/02  | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1444     | 53a     | 6a    | R    | M   | C57    | 11/4/02  | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1451     | 53b     |       | L    | M   | C57    | 11/4/02  | Norm | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1458     | 53c     |       | B    | M   | C57    | 11/4/02  | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1465     | 53d     |       | N    | M   | C57    | 11/4/02  | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1472     | 54      | 6b    | R    | F   | C57    | 11/4/02  | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1479     | 54      |       | L    | F   | C57    | 11/4/02  | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1486     | 54      |       | B    | F   | C57    | 11/4/02  | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1493     | 54      |       | N    | F   | C57    | 11/4/02  | High | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 1500     | 55      | 6a    | R    | F   | C57    | 11/4/02 | High | Yes      | 1    | 1      | 0     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1507     | 55      |       | L    | F   | C57    | 11/4/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1514     | 55      |       | B    | F   | C57    | 11/4/02 | High | Yes      | 1    | 1      | 0     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1521     | 55      |       | N    | F   | C57    | 11/4/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1528     | 56      | 6b    | R    | F   | C3     | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1535     | 56      |       | L    | F   | C3     | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1542     | 56      |       | B    | F   | C3     | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1549     | 56      |       | N    | F   | C3     | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1556     | 57      | 6a    | R    | F   | C3     | 11/4/02 | Low  | Yes      | 1    | 1      | 0     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1563     | 57      |       | L    | F   | C3     | 11/4/02 | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1570     | 57      |       | B    | F   | C3     | 11/4/02 | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1577     | 57      |       | N    | F   | C3     | 11/4/02 | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1584     | 58      | 6b    | R    | M   | C3     | 11/4/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1591     | 58      |       | L    | M   | C3     | 11/4/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1598     | 58      |       | B    | M   | C3     | 11/4/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1605     | 58      |       | N    | M   | C3     | 11/4/02 | Norm | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1612     | 59      | 6a    | R    | M   | C3     | 11/4/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1619     | 59      |       | L    | M   | C3     | 11/4/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1626     | 59      |       | B    | M   | C3     | 11/4/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1633     | 59      |       | N    | M   | C3     | 11/4/02 | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1640     | 60      | 6b    | R    | F   | G3     | 11/4/02 | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1647     | 60      |       | L    | F   | G3     | 11/4/02 | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1654     | 60      |       | B    | F   | G3     | 11/4/02 | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1661     | 60      |       | N    | F   | G3     | 11/4/02 | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1668     | 61      | 6a    | R    | F   | C3     | 11/4/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1675     | 61      |       | L    | F   | C3     | 11/4/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1682     | 61      |       | B    | F   | G3     | 11/4/02 | High | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 1689     | 61      |       | N    | F   | C3     | 11/4/02 | High | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1696     | 62      | 6b    | R    | F   | C57    | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1703     | 62      |       | L    | F   | C57    | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1710     | 62      |       | B    | F   | C57    | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1717     | 62      |       | N    | F   | C57    | 11/4/02 | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1724     | 63a     | 7a    | R    | M   | C57    | 1/8/03  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1731     | 63b     |       | L    | M   | C57    | 1/8/03  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1738     | 63c     |       | B    | M   | C57    | 1/8/03  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1745     | 63d     |       | N    | M   | C57    | 1/8/03  | Low  | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1752     | 64      | 7b    | R    | M   | C57    | 1/8/03  | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1759     | 64      |       | L    | M   | C57    | 1/8/03  | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1766     | 64      |       | B    | M   | C57    | 1/8/03  | Low  | No       | 1    | 1      | 0     | 0     | 0    | 0      | 0     | 1      | 1         | 1       |
| 1773     | 64      |       | N    | M   | C57    | 1/8/03  | Low  | No       | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1780     | 65      | 7a    | R    | F   | C57    | 1/8/03  | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 0       |
| 1787     | 65      |       | L    | F   | C57    | 1/8/03  | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1794     | 65      |       | B    | F   | C57    | 1/8/03  | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 0         | 1       |
| 1801     | 65      |       | N    | F   | C57    | 1/8/03  | Norm | Yes      | 1    | 1      | 0     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 1808     | 66a     | 7b    | R    | F   | C57    | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1815     | 66a     |       | L    | F   | C57    | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1822     | 66a     |       | B    | F   | C57    | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1829     | 66a     |       | N    | F   | C57    | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1836     | 66b     |       | R    | F   | C57    | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1843     | 66b     |       | L    | F   | C57    | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 1850     | 67a     | 7a    | R    | M   | C57    | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1857     | 67b     |       | L    | M   | C57    | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 1       |
| 1864     | 67c     |       | B    | M   | C57    | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1871     | 67d     |       | N    | M   | C57    | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1878     | 68      | 7b    | R    | M   | C57    | 1/8/03  | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1885     | 68      |       | L    | M   | C57    | 1/8/03  | High | No       | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 1       |
| 1892     | 68      |       | B    | M   | C57    | 1/8/03  | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1899     | 68      |       | N    | M   | C57    | 1/8/03  | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1906     | 69      | 7a    | R    | M   | C3     | 1/8/03  | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1913     | 69      |       | L    | M   | C3     | 1/8/03  | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1920     | 69      |       | B    | M   | C3     | 1/8/03  | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1927     | 69      |       | N    | M   | C3     | 1/8/03  | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1934     | 70      | 7b    | R    | M   | C3     | 1/8/03  | Low  | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1941     | 70      |       | L    | M   | C3     | 1/8/03  | Low  | No       | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 1       |
| 1948     | 70      |       | B    | M   | C3     | 1/8/03  | Low  | No       | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 1       |
| 1955     | 70      |       | N    | M   | C3     | 1/8/03  | Low  | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1962     | 71      | 7a    | R    | F   | C3     | 1/8/03  | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1969     | 71      |       | L    | F   | C3     | 1/8/03  | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 1976     | 71      |       | B    | F   | C3     | 1/8/03  | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1983     | 71      |       | N    | F   | C3     | 1/8/03  | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 1990     | 72      | 7b    | R    | F   | C3     | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 1997     | 72      |       | L    | F   | C3     | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2004     | 72      |       | B    | F   | C3     | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2011     | 72      |       | N    | F   | C3     | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2018     | 73      | 7a    | R    | M   | C3     | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 0       |
| 2025     | 73      |       | L    | M   | C3     | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2032     | 73      |       | B    | M   | C3     | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2039     | 73      |       | N    | M   | C3     | 1/8/03  | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2046     | 74a     | 7b    | R    | M   | C3     | 1/8/03  | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2053     | 74a     |       | L    | M   | C3     | 1/8/03  | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2060     | 74a     |       | B    | M   | C3     | 1/8/03  | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2067     | 74a     |       | N    | M   | C3     | 1/8/03  | High | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2074     | 74b     | 7b    | R    | M   | C3     | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 0       |
| 2081     | 74b     |       | L    | M   | C3     | 1/8/03  | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2088     | 75      | 8a    | R    | F   | C57    | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2095     | 75      |       | L    | F   | C57    | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2102     | 75      |       | B    | F   | C57    | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2109     | 75      |       | N    | F   | C57    | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 2116     | 76      | 8b    | R    | F   | C57    | 1/15/03 | Low  | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2123     | 76      |       | L    | F   | C57    | 1/15/03 | Low  | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2130     | 76      |       | B    | F   | C57    | 1/15/03 | Low  | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2137     | 76      |       | N    | F   | C57    | 1/15/03 | Low  | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2144     | 77a     | 8a    | R    | M   | C57    | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2151     | 77b     |       | L    | M   | C57    | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2158     | 77c     |       | B    | M   | C57    | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2165     | 77d     |       | N    | M   | C57    | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2172     | 78      | 8b    | R    | M   | C57    | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2179     | 78      |       | L    | M   | C57    | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2186     | 78      |       | B    | M   | C57    | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2193     | 78      |       | N    | M   | C57    | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2200     | 79      | 8a    | R    | F   | C57    | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2207     | 79      |       | L    | F   | C57    | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2214     | 79      |       | B    | F   | C57    | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2221     | 79      |       | N    | F   | C57    | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2228     | 80      | 8b    | R    | F   | C57    | 1/15/03 | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2235     | 80      |       | L    | F   | C57    | 1/15/03 | High | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2242     | 80      |       | B    | F   | C57    | 1/15/03 | High | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2249     | 80      |       | N    | F   | C57    | 1/15/03 | High | No       | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 0       |
| 2256     | 81      | 8a    | R    | F   | C3     | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2263     | 81      |       | L    | F   | C3     | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2270     | 81      |       | B    | F   | C3     | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 0       |
| 2277     | 81      |       | N    | F   | C3     | 1/15/03 | Low  | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2284     | 82      | 8b    | R    | F   | C3     | 1/15/03 | Low  | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2291     | 82      |       | L    | F   | C3     | 1/15/03 | Low  | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2298     | 82      |       | B    | F   | C3     | 1/15/03 | Low  | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2305     | 82      |       | N    | F   | C3     | 1/15/03 | Low  | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2312     | 83      | 8a    | R    | M   | C3     | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2319     | 83      |       | L    | M   | C3     | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2326     | 83      |       | B    | M   | C3     | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2333     | 83      |       | N    | M   | C3     | 1/15/03 | Norm | Yes      | 1    | 1      | 1     | 0     | 0    | 0      | 0     | 1      | 0         | 0       |
| 2340     | 84a     | 8b    | R    | M   | C3     | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2347     | 84a     |       | L    | M   | C3     | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2354     | 84a     |       | B    | M   | C3     | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2361     | 84a     |       | N    | M   | C3     | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2368     | 84b     |       | R    | M   | C3     | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2375     | 84b     |       | N    | M   | C3     | 1/15/03 | Norm | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2382     | 85      | 8a    | R    | F   | C3     | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2389     | 85      |       | L    | F   | C3     | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2396     | 85      |       | B    | F   | C3     | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2403     | 85      |       | N    | F   | C3     | 1/15/03 | High | Yes      | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet   | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|--------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 2410     | 86a 8b  |       | R    | F   | C3     | 1/15/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2417     | 86a     |       | L    | F   | C3     | 1/15/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 0      | 0     | 1      | 0         | 1       |
| 2424     | 86a     |       | B    | F   | C3     | 1/15/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2431     | 86a     |       | N    | F   | C3     | 1/15/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2438     | 86b     |       | R    | F   | C3     | 1/15/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2445     | 86b     |       | N    | F   | C3     | 1/15/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2452     | 87a 9a  |       | R    | F   | C57    | 3/19/03 | Low    | Yes      | 1    | 1      | 0     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2459     | 88 9b   |       | N    | F   | G57    | 3/19/03 | Low    | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2466     | 89 9a   |       | N    | M   | C57    | 3/19/03 | High   | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2473     | 90 9b   |       | L    | M   | C57    | 3/19/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2480     | 91 9a   |       | B    | M   | C57    | 3/19/03 | Normal | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2487     |         |       | R    | M   | C57    | 3/19/03 | Normal | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2494     | 92a 9b  |       | R    | M   | C3     | 3/17/03 | Low    | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2501     |         |       | L    | M   | G3     | 3/17/03 | Low    | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2508     |         |       | B    | M   | G3     | 3/17/03 | Low    | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2515     | 92b 9b  |       | R    | M   | C3     | 3/17/03 | Low    | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2522     |         |       | L    | M   | G3     | 3/17/03 | Low    | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2529     |         |       | B    | M   | C3     | 3/17/03 | Low    | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2536     | 93 9a   |       | R    | M   | C3     | 3/17/03 | Low    | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2543     |         |       | L    | M   | C3     | 3/17/03 | Low    | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2550     |         |       | B    | M   | C3     | 3/17/03 | Low    | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2557     | 94a 9b  |       | R    | M   | C3     | 3/17/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2564     |         |       | L    | M   | C3     | 3/17/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2571     |         |       | B    | M   | C3     | 3/17/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2578     | 94b 9b  |       | N    | M   | C3     | 3/17/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2585     |         |       | B    | M   | C3     | 3/17/03 | High   | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2592     | 95 9a   |       | R    | M   | C3     | 3/17/03 | High   | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2599     |         |       | L    | M   | C3     | 3/17/03 | High   | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2606     |         |       | B    | M   | C3     | 3/17/03 | High   | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2613     | 97 9a   |       | R    | F   | G3     | 3/17/03 | Normal | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2620     |         |       | L    | F   | C3     | 3/17/03 | Normal | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2627     |         |       | B    | F   | C3     | 3/17/03 | Normal | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2634     |         |       | N    | F   | C3     | 3/17/03 | Normal | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2641     | 98 9b   |       | R    | F   | G3     | 3/17/03 | Normal | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2648     |         |       | L    | F   | G3     | 3/17/03 | Normal | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2655     |         |       | B    | F   | C3     | 3/17/03 | Normal | No       | 1    | 1      | 0     | 1     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2662     |         |       | N    | F   | C3     | 3/17/03 | Normal | No       | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2669     | 99 10a  |       | R    | M   | C57    | 3/26/03 | Low    | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2676     | 101 10a |       | N    | M   | C57    | 3/26/03 | High   | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2683     | 103 10a |       | R    | F   | G57    | 3/26/03 | Low    | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2690     | 105 10a |       | N    | F   | C57    | 3/26/03 | High   | Yes      | 1    | 1      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet   | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|--------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 2697     | 107     | 10a   | R    | M   | C3     | 3/26/03 | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2704     |         |       | L    | M   | C3     | 3/26/03 | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2711     |         |       | B    | M   | C3     | 3/26/03 | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2748     | 408     | 40b   | R    | F   | G3     | 3/24/03 | Low    | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2725     |         |       | L    | F   | G3     | 3/24/03 | Low    | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2732     |         |       | B    | F   | C3     | 3/24/03 | Low    | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2739     |         |       | N    | F   | C3     | 3/24/03 | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2746     | 409     | 40a   | R    | F   | G3     | 3/24/03 | Low    | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2753     |         |       | L    | F   | G3     | 3/24/03 | Low    | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2760     |         |       | B    | F   | G3     | 3/24/03 | Low    | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2767     | 440     | 40b   | R    | F   | G3     | 3/24/03 | High   | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2774     |         |       | L    | F   | G3     | 3/24/03 | High   | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2781     |         |       | B    | F   | C3     | 3/24/03 | High   | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2788     |         |       | N    | F   | C3     | 3/24/03 | High   | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2795     | 111     | 10a   | R    | F   | C3     | 3/24/03 | High   | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2802     |         |       | L    | F   | C3     | 3/24/03 | High   | Yes      | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 2809     |         |       | B    | F   | C3     | 3/24/03 | High   | Yes      | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 0      | 1         | 0       |
| 2816     |         |       | N    | F   | C3     | 3/24/03 | High   | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2823     | 113     | 11a   | R    | M   | C57    | 6/4/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2830     |         |       | L    | M   | C57    | 6/4/03  | Low    | Yes      | 0    | 0      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2837     |         |       | N    | M   | C57    | 6/4/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2844     | 115     | 11a   | R    | M   | C57    | 6/4/03  | High   | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2851     |         |       | L    | M   | C57    | 6/4/03  | High   | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2858     |         |       | N    | M   | C57    | 6/4/03  | High   | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2865     | 117     | 11a   | R    | F   | C57    | 6/11/03 | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2872     |         |       | L    | F   | C57    | 6/11/03 | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2879     |         |       | B    | F   | C57    | 6/11/03 | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2886     |         |       | N    | F   | C57    | 6/11/03 | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2893     | 119     | 11a   | R    | M   | C3     | 6/2/03  | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2900     |         |       | N    | M   | G3     | 6/2/03  | Normal | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2907     | 120     | 11b   | R    | M   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2914     |         |       | L    | M   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2921     |         |       | N    | M   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 1         | 0       |
| 2928     | 121     | 11a   | R    | M   | C3     | 6/2/03  | High   | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2935     |         |       | N    | M   | G3     | 6/2/03  | High   | Yes      | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 2942     | 122     | 11b   | R    | M   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2949     |         |       | L    | M   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2956     |         |       | N    | M   | C3     | 6/2/03  | Low    | No       | 0    | 0      | 0     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2963     | 123     | 11a   | R    | F   | C3     | 6/2/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2970     |         |       | L    | F   | C3     | 6/2/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 0     | 1      | 0         | 1       |
| 2977     |         |       | N    | F   | C3     | 6/2/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |

| Mouse ID | Cage ID | Group | Mark | Sex | Strain | DOB     | Diet   | Exercise | Scan | Analy. | Mech. | Raman | Scan | Analy. | Mech. | Analy. | Histology | Re-fill |
|----------|---------|-------|------|-----|--------|---------|--------|----------|------|--------|-------|-------|------|--------|-------|--------|-----------|---------|
| 2984     | 124     | 11b   | R    | F   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 2991     |         |       | L    | F   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 1       |
| 2998     |         |       | B    | F   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 3005     |         |       | N    | F   | C3     | 6/2/03  | Low    | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 3012     | 125     | 11a   | R    | F   | C3     | 6/2/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 3019     |         |       | L    | F   | C3     | 6/2/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 3026     |         |       | N    | F   | C3     | 6/2/03  | Low    | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 3033     | 127     | 11a   | R    | F   | C3     | 6/2/03  | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 3040     |         |       | N    | F   | C3     | 6/2/03  | Normal | Yes      | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 0      | 1         | 0       |
| 3047     | 128     | 12b   | N    | F   | C3     | 6/16/03 | Normal | No       | 0    | 0      | 0     | 0     | 0    | 0      | 0     | 0      | 0         | 0       |
| 3054     | 130     | 12b   | R    | F   | C3     | 6/16/03 | High   | No       | 1    | 1      | 1     | 0     | 0    | 1      | 1     | 1      | 0         | 0       |
| 3061     |         |       | L    | F   | C3     | 6/16/03 | High   | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
| 3068     |         |       | N    | F   | C3     | 6/16/03 | High   | No       | 1    | 1      | 1     | 0     | 1    | 1      | 1     | 1      | 1         | 0       |
|          |         |       |      |     |        |         |        |          | 392  | 392    | 128   | 30    | 382  | 381    | 393   | 392    | 120       | 122     |

## INFLUENCES OF NUTRITION AND PHYSICAL FORCES ON BONE STRUCTURE/FUNCTION PROPERTIES

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## INTRODUCTION

Maintenance of bone integrity is dependent on a complex interaction of metabolic (hormones, cytokines, growth factors) and environmental factors (mechanical forces, nutrition availability)<sup>1</sup>. Unfortunately, the specific relationships between these factors and the biomechanical properties of bone tissue remain incompletely quantified. Understanding the influence of nutritional status and mechanical usage on the properties of bone may enhance our ability to prevent stress fractures associated with intense training or fragility fractures accompanying aging bone loss.

The purpose of this study was to investigate the interaction between calcium metabolism and exercise mediated mechanical load on the biomechanical properties of bone.

## METHODS

C57BL/6J (n=84 male, n=90 female) and C3H/HeJ (n=69 male, n=67 female) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and separated into groups based on strain, gender, diet, and exercise regime. A synthetic diet consisting of low (0.02% Ca), normal (0.95% Ca), and high (2.0% Ca) basal feed with 10% lactose and 0.67% phosphorus was purchased from Purina Test Diet (Richmond, IN)<sup>2</sup>. An exercise protocol was established and implemented, which consisted of running the mice on a custom built treadmill with a 0° incline. The eight week regime consisted of ramping the speed from 10 to 17 m/min and increasing the duration from 15 to 30 min over the first 4 wks and holding the speed constant for an additional 4 wks<sup>3</sup>. The exercise regime and the controlled calcium diets (low, normal, high) were begun when the animals were 12 weeks old. The mice were humanely euthanized at 20 wks of age, the left femur and eighth caudal vertebrae were dissected free of soft tissue and frozen in LRS. This work was approved by The Animal Care and Use Committee.

Left femora and Cd8 vertebrae were scanned in distilled water on a GE.EVS Micro-CT system. Each scan was reconstructed at a mesh size of 18µm x 18µm x 18µm and a 3D digitized image was generated for each specimen. The femora and vertebrae images were rotated into a standard orientation and thresholded<sup>4</sup>. Geometric analyses were performed on a 3mm mid-diaphysis segment in order to obtain the cross-sectional area, cortical thickness, and moment of inertia (I<sub>yy</sub>) for each femur. Two standardized volumes of trabecular bone were segmented from the proximal and distal ends of the vertebral bodies for analysis. The bone volume fraction, bone surface to volume ratio, trabecular thickness, trabecular number, trabecular spacing, and the degree of anisotropy were acquired from the vertebral morphologic analyses.

The data was analyzed using SPSS statistical software (Chicago, IL). Multivariate general linear models were used to test for main effects of four factors (gender, strain, diet and exercise) and for interaction effects among the four factors. *Post hoc* tests were used to compare the three levels of dietary calcium. Correlations were considered significant with a p-value less than 0.05.

## RESULTS

**Femora:** Geometric data was collected from 323 femora. Mouse strain had a significant effect on geometric parameters. C57BL/6J mice had a significantly smaller cross-sectional area and cortical thickness as compared to the C3H/HeJ mice. Exercise did not have a significant effect on any of the geometric parameters, as shown in Table 1. Table 2 shows that differences in micro-CT measures by dietary calcium level are not significant.

Table 1: Exercise Effects on Cortical Parameters

| Exercise | N   | Cross Sectional Area (mm <sup>2</sup> ) | Cortical Thickness (mm) | Bending I <sub>yy</sub> (mm <sup>4</sup> ) |
|----------|-----|-----------------------------------------|-------------------------|--------------------------------------------|
| No       | 168 | 0.892 (0.20)                            | 0.256 (0.08)            | 0.116 (0.03)                               |
| Yes      | 155 | 0.877 (0.20)                            | 0.250 (0.08)            | 0.114 (0.02)                               |

Data are given as mean (STD)

Table 2: Dietary Effects on Cortical Parameters

| Dietary Calcium | N   | Cross Sectional Area (mm <sup>2</sup> ) | Cortical Thickness (mm) | Bending I <sub>yy</sub> (mm <sup>4</sup> ) |
|-----------------|-----|-----------------------------------------|-------------------------|--------------------------------------------|
| Low             | 105 | 0.891 (0.19)                            | 0.252 (0.08)            | 0.119 (0.03)                               |
| Normal          | 112 | 0.884 (0.20)                            | 0.254 (0.08)            | 0.114 (0.02)                               |
| High            | 106 | 0.878 (0.21)                            | 0.254 (0.08)            | 0.112 (0.02)                               |

Data are given as mean (STD)

**Vertebrae:** Morphologic data was collected from 310 vertebrae.

There was no significant effect for exercise on the trabecular parameters, as shown in Table 3. The differences in proximal trabecular parameters for the three dietary calcium groups are shown in Table 4. The normal dietary calcium group had a significantly higher bone volume fraction as compared to the low (p=0.003) and high (p=0.003) groups. The bone surface to volume ratio of the normal group was shown to be significantly lower than the low dietary calcium group (p=0.030), however no significant differences were seen between any of the other groupings. The normal dietary calcium group had a significantly higher trabecular thickness as compared to the low group (p<0.000) and marginally significant as compared to the high (p=0.051) group. Similar results were observed in the distal trabecular parameters.

Table 3: Exercise Effects on Proximal Trabecular Parameters

| Exercise | N   | Bone Volume Fraction (%) | Bone Surface to Volume Ratio (mm <sup>2</sup> /mm <sup>3</sup> ) | Trabecular Thickness (mm) |
|----------|-----|--------------------------|------------------------------------------------------------------|---------------------------|
| No       | 161 | 40.9 (0.07)              | 30.08 (4.57)                                                     | 0.074 (0.01)              |
| Yes      | 149 | 41.9 (0.06)              | 29.30 (4.44)                                                     | 0.077 (0.01)              |

Data are given as mean (STD)

Table 4: Dietary Effects on Proximal Trabecular Parameters

| Dietary Calcium | N   | Bone Volume Fraction (%) | Bone Surface to Volume Ratio (mm <sup>2</sup> /mm <sup>3</sup> ) | Trabecular Thickness (mm) |
|-----------------|-----|--------------------------|------------------------------------------------------------------|---------------------------|
| Low             | 101 | 40.6 (0.07)*             | 30.29 (4.01)*                                                    | 0.073 (0.01)*             |
| Normal          | 109 | 42.8 (0.07)*             | 29.18 (5.26)*                                                    | 0.078 (0.02)*             |
| High            | 100 | 40.6 (0.06)*             | 29.69 (4.07)*                                                    | 0.075 (0.01)*             |

Data are given as mean (STD) \* Significant, p ≤ 0.05

## DISCUSSION

As expected, the results indicate that geometric properties are dependent on genetic background. C57BL/6J mice had a significantly smaller cross-sectional area and cortical thickness as compared to the C3H/HeJ mice, as noted in previous studies<sup>5</sup>.

Differences due to nutritional status were seen only in trabecular bone of the vertebrae, where modeling phenomena are expected to occur more rapidly than in femoral cortical bone due to the greater biodynamic activity of trabecular bone. The loading regimen in this study appears to not have an effect on the femoral geometric or vertebral morphologic properties. The level of exercise did not compensate for the decrease in the trabecular parameters during dietary calcium alterations. This may be caused by an inadequate load stimulus. Preliminary biomechanical testing data suggests a difference in post-yield behavior in exercise groups. This may possibly suggest an interaction at the tissue level, which we will continue to explore.

## REFERENCES

1. Silbermann et al. *Calcif Tissue Int*, 46: 80-93, 1990.
2. Cromphaut et al. *PNAS*, 98: 13324-13329, 2001.
3. Kohut et al. *Mech Ageing Dev*, 122: 1135-1150, 2001.
4. Kuhn et al. *J Orthop Res*, 8: 833-842, 1990.
5. Chen et al. *Bone*, 25: 413-420, 1990.

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